

Visual Cues Enhance the Response of *Lygus hesperus* (Heteroptera: Miridae) to Volatiles from Host Plants

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ABSTRACT Studies were conducted to examine the behavioral response of fifth-instar and adult *Lygus hesperus* Knight (Heteroptera: Miridae) to odors associated with a host plant (alfalfa) when presented singly or in combination with a visual plant cue (530 nm, green light-emitting diode [LED]). Bioassays were conducted in a modified Y-tube olfactometer, where incoming air was filtered and humidified before passing through chambers that held plant/conspecific treatments. A LED, placed behind an organdy screen at the end of one arm of the Y-tube, simulated a visual plant cue. In agreement with our previous findings, fifth-instar and adult female *L. hesperus* were attracted to plant/conspecific odor combinations, but adult males were not. Independently, the LED also was attractive to immature and adult female *L. hesperus*, and for all life stages, a much larger percentage of the bugs walked to the extreme end of the Y-tube when the visual cue was present. When visual and volatile cues were presented simultaneously, responses by both immature and adult (male and female) *L. hesperus* were significantly enhanced. Plant/conspecific treatments that had been marginally acceptable when only volatile stimuli were available showed the greatest enhancement in response with the addition of the visual cue. Presentation of visual and volatile cues together led to a mean increase in the percentage of bugs that walked to the extreme end of the Y-tube (nymphs, 36.6%; females, 23.4%; males, 26.1%). Results are discussed in terms of stage-specific responses and how these behavioral differences will likely play a role in developing effective trapping and monitoring systems for this important pest.

KEY WORDS light-emitting diode, olfactory cues, western tarnished plant bug, additive response, synergistic response

THE WESTERN TARNISHED PLANT bug, *Lygus hesperus* Knight (Heteroptera: Miridae), is a polyphagous herbivore that can cause severe damage in a number of economically important crops (Strong 1970, Leigh 1976, Mauney and Henneberry 1984, Leigh et al. 1988, Ellsworth 2000). This species is found throughout western North America, and over the last several years, it has been rated as the number one pest of cotton in Arizona. Despite an average of 4.4 applications of conventional pesticides per year, at a cost of \$55/acre, losses in excess of \$17.2 mil/yr have been estimated (Ellsworth and Barkley 2001). Heavy reliance on traditional, broad-spectrum insecticides for control of these pests has resulted in resistance in a number of cropping systems (Xhu and Brindley 1992, Grafton-Cardwell et al. 1997), as well as led to disruption of biological control agents (Udayagiri et al. 2000). An ecologically sound and sustainable manage-

ment system for controlling *L. hesperus* is urgently needed.

One economically acceptable alternate control strategy for *L. hesperus* would use sex pheromones. Unfortunately, after nearly three decades of work in this area, field trials of potential attractants have been unsuccessful (Aldrich et al. 1988, Millar et al. 2000, Ho and Millar 2002). Another approach that has been useful for monitoring various other agriculturally important insect pests involves the identification of pertinent plant volatile and/or visual cues (Muirhead-Thomson 1991, Metcalf and Metcalf 1992). One of the best studied examples of the latter phenomenon is the apple maggot, *Rhagoletis pomonella* (Walsh), for which synthetic fruit volatiles in combination with a visual cue simulating the red apple have been used for monitoring and control purposes (Prokopy et al. 1987, 1990, Rull and Prokopy 2003, 2005). In *L. hesperus*, host location and selection behaviors are poorly understood. Previously, Blackmer et al. (2004) showed that odors emanating from alfalfa and alfalfa + *Lygus* conspecific combinations mediated host-location behaviors in *L. hesperus*. However, the upwind response was much more pronounced for nymphs than for females,

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and males were apparently not attracted to alfalfa odors in the context of these experiments. We speculated that additional cues (i.e., visual or the combination of visual and volatile cues) might be needed to elicit a more complete response. Landis and Fox (1972) previously showed that *L. hesperus* was strongly attracted to visual cues simulating a host plant, and for the closely related *Lygus lineolaris* (Palisot de Beauvois), their response to visual cues has been exploited in a number of trapping systems (Prokopy et al. 1979, 1982, Boivin et al. 1982, Boivin and Stewart 1984, Rancourt et al. 2000). Here we studied the role and relative importance of volatile and visual plant cues on upwind orientation behavior of *L. hesperus*. A modified Y-tube olfactometer allowed us to present cues singly or simultaneously. Results are discussed in relation to host finding differences between generalist and specialist herbivores and subsequent implications to integrated pest management (IPM) programs.

Materials and Methods

Insect Rearing and Maintenance. *Lygus hesperus* nymphs and adults were collected from alfalfa fields located at The University of Arizona-Maricopa Agricultural Center, Maricopa, AZ. To maintain genetic diversity, feral individuals were added to the colony three to four times per year. Green beans, carrots, pink bollworm eggs [*Pectinophora gossypiella* (Saunders)], and 10% sucrose solution were provided as food. The green beans and carrots also served as oviposition substrates. Food was changed every other day, and the previously used beans and carrots were placed in 2 by 14-cm-diameter petri dishes that were lined with filter paper and maintained in an incubator until first-instar *L. hesperus* emerged. Newly emerged nymphs were placed in 8.5 by 12.5-cm-diameter paper cartons where the center of each lid had been replaced with nylon organdy to allow air circulation. Nymphs and adults were provided food and a 10% sucrose solution until they were needed for the experiments. Insects were maintained in an incubator at $23 \pm 2^\circ\text{C}$, $55 \pm 15\%$ RH, and under a light-dark regimen of 14:10 (L:D) h.

Plant Maintenance. *Medicago sativa* L. (cultivar Cuf 101) was planted in 1-liter pots containing a standard potting soil mixture and maintained in a greenhouse at $25 \pm 5^\circ\text{C}$ and 50–85% RH. Natural lighting provided a light-dark regimen of 12:12 (L:D) h. Plants were watered and fertilized regularly by means of a drip irrigation system. A 1:1 mixture of all-purpose Scotts Miracle-Gro Excel (21-5-20) and cal-mag Miracle-Gro Professional (15-5-15) was applied at a rate of 1/100 liters of water.

Y-Tube Setup. Bioassays were conducted in a 40-mm-diameter \times 36-cm-long glass Y-tube olfactometer that had a 50° inside angle and an inside arm length of 12 cm. Incoming air was filtered through activated charcoal and humidified with distilled water. The filtered air was split between two 2-liter holding chambers: one chamber served as a control (clean air) and the other held the plant/conspecific

material. From each holding chamber, the air moved into the arms of the Y-tube and through an organdy screen before entering the main tube of the olfactometer. Airflow through the system was maintained at 4.0 liters/min (3.2 m/min inside the tube) by an inline flow meter (Gilmont Instruments; Barnant Co., Barrington, IL). A smoke test showed a steady, laminar air flow in both arms and throughout the olfactometer.

A 60-cm-long, wide-spectrum fluorescent lamp (GE, F20T12-PL/AQ) was positioned 22 cm above the arms of the Y-tube. Before each trial, light intensity over each arm was measured with a light meter (ExTech Instruments Model 401025; Zefon International, St. Petersburg, FL), and the tube was adjusted until intensity was the same in both arms. Light intensity averaged 704.6 ± 15.1 (SE) lux during the bioassays. The Y-tube setup was surrounded by a 50 by 70 by 60-cm wooden enclosure painted flat black. The holding chambers were placed outside this enclosure to eliminate visual cues from the plant material. A light-emitting diode (Green LED; NSPG520S; Nichia America, Mountville, PA) was used to simulate a visual plant cue. The light emitted a narrow wavelength in the range of 530 nm, and power was supplied by a universal adapter that provided 6 V DC. The LED was inserted behind an organdy screen at the far upwind end of the Y-tube setup and was flush with the side wall of the tube to eliminate turbulence in the air flow.

Bioassays. Approximately 30 min to 1 h before trials were initiated, fifth-instar or 7- to 10-d-old adult *L. hesperus* were placed into individual holding/release tubes. Each tube was constructed from a 15.5-cm-long, 5.8-ml disposable pipette (TX20403; A. Daigger & Company, Wheeling, IL) from which 0.5 cm of the bulb and 8 cm of the pipette tip were removed. The cut end of the pipette tip was covered with organdy. A nymph or adult was placed inside the tube, and the end where the bulb tip had been removed was sealed with a cork. Tubes containing bugs were placed into a separate holding container, so they would not be exposed to treatments before their release. Previous experiments (Blackmer et al. 2004) showed that the response of experienced and naïve *L. hesperus* to plant volatiles was similar; thus, only naïve individuals were used in this study.

At the beginning of each trial, the cork was removed from the holding/release tube, and the open end was placed at the downwind end of the Y-tube. Each insect was given 5 min to respond, and a choice for the left or right arm of the olfactometer was noted when the insect was 1 cm past the Y junction. The variables recorded were percentage of bugs exiting the holding tube, time required for bugs to exit, percentage of bugs walking upwind, time needed to choose between arms, percentage of bugs responding to treatment, and percentage of bugs walking to the extreme end of the Y-tube. Treatments included a blank air control, plant or plant + conspecific cues alone, a visual cue alone, and a visual cue in combination with the two most attractive and the two least attractive treatments from a previous study (Blackmer et al. 2004). The treat-

Table 1. Treatments tested with and without the addition of a visual cue (LED)

Stage	Best response to treatment	Worst response to treatment
Nymphs	Flowering alfalfa + conspecifics (FA + C) Vegetative alfalfa + feeding damage (VA + FD)	Flowering alfalfa + feeding damage (FA + FD) Flowering alfalfa + feeding damage + conspecifics (FA + FD/C)
Females	Flowering alfalfa + feeding damage (FA + FD) Vegetative alfalfa + conspecifics (VA + C)	Flowering alfalfa + conspecifics (FA + C) Vegetative alfalfa (VA)
Males	Vegetative alfalfa + conspecifics (VA + C) —	Vegetative alfalfa (VA) Flowering alfalfa (FA)

Selection based on best and worst responses by *L. hesperus* nymphs and adults from a previous study (Blackmer et al. 2004).
—, no attractive host was found for males, so only the single “best” treatment was tested

ments were selected to cover the extremes in responses when only volatile cues were presented. Treatments and abbreviations are listed in Table 1.

For vegetative alfalfa treatments, we used 30-cm-tall, intact plants in which their root systems were wrapped with moist paper towels and enclosed inside a plastic sleeve. For flowering alfalfa, we used five stems, which were cut 30 cm below the flowers and wrapped as above. This was necessary because flowering alfalfa plants were too large (0.5 m³) to fit inside the holding chambers. Headspace volatile profiles of these excised alfalfa treatments were comparable (in terms of compounds detected and relative amounts) compared with headspace volatiles from intact plant collections from the greenhouse, at least during the 1-h time span in which they were used (Blackmer et al. 2004; J.L.B., unpublished data). For the treatments that consisted of plants plus conspecifics, we used a 1:1 sex ratio of ≈10 adults or ≈10 similar aged nymphs, when adults and nymphs were tested, respectively. Mixed sexes were used to simulate conditions that *Lygus* bugs encounter under field conditions. For treatments with nymphs, we used only nymphs as they tend to be aggregated in the field and may or may not be associated with the more mobile adults (J.L.B., unpublished data). For 24- to 48-h feeding damage treatments, we placed ≈10–20 adults (1:1 sex ratio) or nymphs inside fine-mesh bags on the plants that were to be tested. Fresh plant material was placed in the holding chamber every hour, and the treatment and control chambers were switched. This eliminated any potential bias caused by odor source location.

Bioassays were conducted between 1000 and 1800 hours using insects that were tested only once. For each individual, a clean Y-tube was used, and on a given day, ≈20 individuals were tested. Tests continued until ≈40 individuals per treatment had walked upwind and selected either the right or left arm of the Y-tube. However, because not all insects exited the release tubes, walked upwind, or chose between arms of the Y-tube, we had to test 938 nymphs, 630 females, and 386 males to obtain a sufficient end response. During the assays, temperature was maintained at 25.9 ± 0.7°C and ambient relative humidity averaged 38.5 ± 9.3%.

Statistics. The null hypothesis that *L. hesperus* showed no preference for either olfactometer arm (a response equal to 50:50) was analyzed with a χ^2 goodness-of-fit test after correcting for continuity with Yates' correction factor (Zar 1984). Trials with and

without the visual cue were compared by two-way contingency tables. Time required for nymphs and adults to exit the release tube, percentage of bugs leaving the release tube, percentage that walked upwind to an arm, response time to first choice, and percentage that walked all the way to the end of the Y-tube for controls versus treatments were compared by two-way ANOVAs. Percent data were transformed by the arcsine function, and exit time and time to first choice data by the logarithmic function when needed to meet the requirements of normality and homogeneity of variance before analyses. Herein, additive response refers to results where the sum of the responses to both plant cues (visual and volatile) was equal to or less than the individual cues when presented alone (i.e., no more than a two-fold increase in attraction), while synergistic response refers to results where the combined effect of the plant cues acting together was greater than the sum of the cues when presented alone (i.e., more than a two-fold increase in attraction).

Results

Nymphal Response to Volatile and Visual Plant Cues. When *L. hesperus* nymphs were presented alfalfa and/or conspecific volatile cues, a preference to odors associated with flowering alfalfa with conspecifics (FA + C; $\chi^2 = 17.4$, $P < 0.001$), and to vegetative and flowering alfalfa that had been fed on for 24–48 h (VA + FD; $\chi^2 = 15.8$, $P < 0.001$; FA + FD; $\chi^2 = 7.2$, $P < 0.01$; Fig. 1A) was shown (see Table 1). No preference was evident for feeding-damaged flowering alfalfa with conspecifics (FA + FD/C; $\chi^2 = 1.2$, $P > 0.05$) or for the clean air control ($\chi^2 = 0.02$, $P > 0.05$; Fig. 1A). A relatively small percentage of nymphs that walked upwind and made a choice walked all the way (ATW; Fig. 1A, black bars) to the end of the Y-tube (9.9 ± 2.4% [SE]), and even fewer walked ATW to the control side (4.5 ± 1.5%).

When the green LED was presented in combination with the same plant/conspecific treatments, all treatments were preferred over clean air ($P < 0.05$ in all cases; Fig. 1B). The two least attractive treatments when only volatile stimuli were available for making a choice (FA + FD and FA + FD/C) had the greatest response enhancement with the addition of the visual cue (12.8% increase in response). There also was a significant response to the visual cue (LED) in the absence of any volatile cues ($\chi^2 = 5.0$, $P < 0.05$).

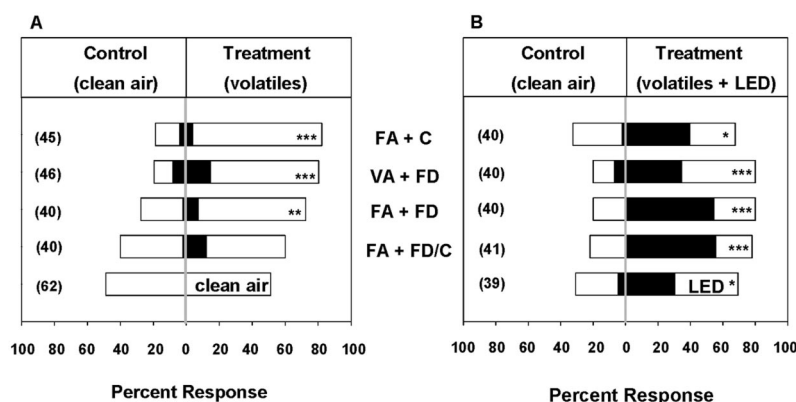


Fig. 1. Response of fifth-instar *L. hesperus* (A) to alfalfa or conspecific odors or clean air and (B) to alfalfa or conspecific odors + an LED (simulated visual plant cue) or LED alone. Black bars represent the percentage of nymphs that walked ATW to the end of the Y-tube. FA + C, flowering alfalfa + conspecifics; VA + FD, vegetative alfalfa where nymphs had fed for 24–48 h; FA + FD, flowering alfalfa where nymphs had fed for 24–48 h; FA + FD/C, flowering alfalfa with feeding damage and conspecifics. ** $P < 0.01$, *** $P < 0.001$; N is the number of insects that walked upwind and made a choice.

Two-way contingency tests comparing the response of nymphs to the LED versus volatile cues only and the LED versus visual + volatile cue combinations were not significant ($P > 0.05$). However, a much larger percentage of nymphs walked ATW to the end of the Y-tube with the visual + volatile cue combinations ($46.5 \pm 5.3\%$; Fig. 1B); a mean increase of 36.6% relative to the response to volatile cues alone. Only $2.5 \pm 1.8\%$ of the nymphs walked ATW to the control side. Approximately 31% of the nymphs walked ATW to the visual cue alone.

Female Response to Volatile and Visual Plant Cues. When females were presented alfalfa and/or conspecific volatile cues, a preference to odors associated with vegetative alfalfa with conspecifics (VA + C; $\chi^2 = 4.8$, $P < 0.05$) and with flowering alfalfa that had been fed on for 24–48 h (FA + FD; $\chi^2 = 6.6$, $P < 0.05$) was shown (Fig. 2A). No significant attraction was

found for vegetative alfalfa (VA; $\chi^2 = 0.6$, $P > 0.05$), for flowering alfalfa with conspecifics (FA + C; $\chi^2 = 2.6$, $P > 0.05$), or for the clean air control ($\chi^2 = 0.02$, $P > 0.05$; Fig. 2A). Approximately $13.6 \pm 4.0\%$ of the females walked ATW to the end of the Y-tube on the treatment side, while only $4.8 \pm 1.1\%$ walked ATW to the end on the control side (Fig. 2A, black bars).

With the addition of the LED, females preferred all plant and plant + conspecific treatments over clean air ($P < 0.05$ in all cases; Fig. 2B). Similar to the nymphs, the two least attractive treatments when only volatile stimuli were available for making a choice (VA and FA + C) had the greatest response enhancement with the addition of the visual cue (34.3% increase). Two-way contingency tests for these two treatments were significant (VA versus VA + visual cue; $\chi^2 = 7.9$, $P < 0.01$; FA + C versus FA + C + visual cue; $\chi^2 = 8.52$, $P < 0.01$). There also was a significant response to the

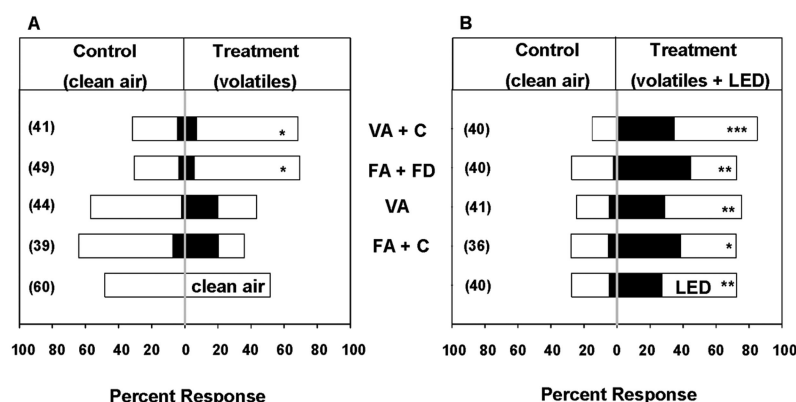


Fig. 2. Response of *L. hesperus* females (A) to alfalfa or conspecific odors or clean air and (B) to alfalfa or conspecific odors + an LED (simulated visual plant cue) or LED alone. Black bars represent the percentage of females that walked ATW to the end of the Y-tube. VA + C, vegetative alfalfa with conspecifics; FA + FD, flowering alfalfa where adults had fed for 24–48 h; VA, vegetative alfalfa; FA + C, flowering alfalfa + conspecifics. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; N is the number of insects that walked upwind and made a choice.

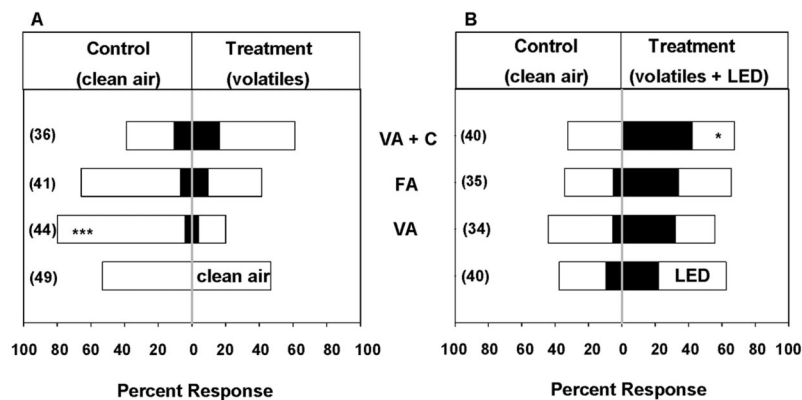


Fig. 3. Response of *L. hesperus* males (A) to alfalfa or conspecific odors or clean air and (B) to alfalfa or conspecific odors + an LED (simulated visual plant cue) or LED alone. Black bars represent the percentage of males that walked ATW to the end of the Y-tube. VA + C, vegetative alfalfa with conspecifics; FA, flowering alfalfa; VA, vegetative alfalfa. * $P < 0.05$, *** $P < 0.001$; N is the number of insects that walked upwind and made a choice.

visual cue in the absence of any volatile cues ($\chi^2 = 7.2$, $P < 0.01$), and two-way contingency tests comparing the LED versus volatile cues for the two worst treatments (VA and FA + C) were significant ($\chi^2 = 10.2$, $P < 0.01$). Contingency tests comparing the LED versus visual + volatile cue combinations were not significant ($P > 0.05$). However, a much larger percentage of females walked ATW to the end of the Y-tube with the visual + volatile combinations ($37.0 \pm 3.3\%$); a mean increase of 23.4% relative to the response to volatile cues alone. Only $3.2 \pm 1.3\%$ of the females walked ATW to the end on the control side. Approximately 27.5% of the females walked ATW with just the visual cue.

Male Response to Volatile and Visual Plant Cues. Males showed no preference to alfalfa with or without conspecific odors, and in fact, showed a strong repelency to vegetative alfalfa (VA; $\chi^2 = 15.0$; $P < 0.001$; Fig. 3A). Approximately $10.3 \pm 3.6\%$ of the males walked ATW to the end of the Y-tube on the treatment

side, whereas $8.9 \pm 2.7\%$ walked ATW to the end on the control side.

With the addition of the LED, males showed a preference to vegetative alfalfa with conspecifics over clean air (VA + C; $\chi^2 = 4.2$; $P < 0.05$), and vegetative alfalfa was no longer repellent (VA; $\chi^2 = 0.3$; $P > 0.05$; Fig. 3B). Similar to nymphs and females, the two least attractive treatments when only volatile stimuli were available (FA and VA), had the greatest response enhancement with the addition of the visual cue (30.0% increase). Two-way contingency tests for these two treatments were significant (FA versus FA + visual cue; $\chi^2 = 3.54$, $P < 0.05$; VA versus VA + visual cue; $\chi^2 = 9.5$, $P < 0.01$). Males did not respond significantly to the visual cue alone ($\chi^2 = 2.0$, $P > 0.05$). However, two-way contingency tests comparing the LED versus volatile cues for the two worst treatments (FA and VA) were significant ($\chi^2 = 10.5$, $P < 0.01$). Contingency tests comparing the LED versus visual + volatile cue combinations were not significant ($P >$

Table 2. Number of fifth-instar and adult *L. hesperus* tested, mean percentage exiting the release tube, exit time (s), percentage walking upwind, time to first choice (s), and percentage walking ATW to the end of the arm of the Y-tube when presented with clean air, a volatile cue, a visual cue, or a visual + volatile cue combination

Treatment	No. tested	Exiting (%)	Time to exit (s)	Upwind (%)	Time to choice (s)	ATW (%)
Nymphs						
Clean air	154	83.1a	52.7bc	48.4bc	177.3a	0a
Volatile cue	419	88.6a	36.8a	42.4a	165.2a	9.9b
Visual cue	95	75.8a	62.4c	41.0bc	146.8a	30.8b
Combination	270	89.2a	40.9ab	62.7c	139.9a	46.5c
Females						
Clean air	81	97.5a	25.6ab	75.0a	108.3a	0a
Volatile cue	214	98.6a	16.0a	82.3ab	89.0a	13.6b
Visual cue	44	100a	11.9a	90.9b	89.5a	27.5b
Combination	291	94.4a	36.8b	83.2ab	104.6a	37.0c
Males						
Clean air	62	96.8a	14.1a	81.7ab	70.4a	0a
Volatile cue	154	97.3a	16.7a	79.5a	78.4a	10.3b
Visual cue	50	96.0a	17.1a	80.0a	90.7a	22.5b
Combination	120	98.3a	15.5a	91.6b	55.5a	36.4c

Means within columns followed by the same capital or lowercase letter are not significantly different for insect stage and treatment, respectively, Two-way ANOVAs followed by Tukey studentized range test, $P > 0.05$.

Table 3. Two-way ANOVAs for percentage exiting release tube, (s), exit times, percentage that walked upwind, time to first choice (s), and percentage that walked ATW to the end of the Y-tube for fifth-instar, female, and male *L. hesperus*

Source of variation	df	Mean square	F ratio	P
Treatment ^a	3	0.021	0.741	0.530
Stage ^b	2	1.166	40.96	<0.001
Interaction	6	0.045	1.58	0.158
Residual	120	0.028	—	—
Total	131	0.046	—	—
Treatment ^a	3	587	2.82	0.042
Stage ^b	2	11,073	53.26	<0.001
Interaction	6	1,016	4.89	<0.001
Residual	120	208	—	—
Total	43	402	—	—
Treatment ^a	3	0.172	4.57	0.005
Stage ^b	2	2.363	62.79	<0.001
Interaction	6	0.144	3.82	0.002
Residual	120	0.038	—	—
Total	131	0.086	—	—
Treatment ^a	3	1,763	1.88	0.137
Stage ^b	2	70,607	75.30	<0.001
Interaction	6	1,665	1.78	0.110
Residual	120	938	—	—
Total	131	2,273	—	—
Treatment ^a	3	3.750	35.06	<0.001
Stage ^b	2	0.049	0.45	0.636
Interaction	6	0.073	0.68	0.667
Residual	120	0.0322	—	—
Total	131	0.0530	—	—

Bold type indicates significant treatment effects ($P < 0.05$).

^a Treatment refers to clean air, volatile cue, visual cue, and the visual + volatile cue combinations.

^b Stage refers to fifth-instar or adult (male and female) *L. hesperus*.

0.05). A much larger percentage of males walked ATW to the end of the Y-tube with the visual + volatile cue combinations ($36.4 \pm 3.1\%$); a mean increase of 26.1% relative to the response to volatile cues alone (Fig. 3B, black bars). Only $3.9 \pm 1.9\%$ of the males walked ATW to the end on the control side. Approximately 22.5% of the males walked ATW with just the visual cue.

Upwind Orientation Behaviors. In terms of orientation responses during the bioassays, a higher percentage of adults exited the holding tubes compared with nymphs (Table 2), but the treatment did not influence this percentage, nor was there a significant insect stage \times treatment interaction (Table 3). Nymphs took longer to exit the release tubes than adults, but males and females did not differ in exit time (Table 2). When grouped across insect stage, exit time differed among treatments, with exit time being faster in the presence of volatile cues alone (Table 3). This was mostly caused by the faster exit time for nymphs. There also was a significant treatment \times insect interaction, where for all treatments except the visual + volatile cue combinations, adults exited faster than nymphs; females were slower to respond to the combination of cues. Fewer nymphs walked upwind compared with adults, but males and females did not differ in terms of upwind movement (Tables 2 and 3). When grouped across insect stage, there was a significant treatment effect (Table 3). A larger percentage of insects walked upwind in the presence of the visual + volatile cue combinations compared with the volatile cue when presented alone. There also was a significant

treatment \times insect interaction, where adults always walked upwind more frequently than nymphs, but in the presence of visual cues, more females than males walked upwind and in the presence of visual + volatile cue combinations more males than females walked upwind. For time required to choose an arm of the Y-tube, insect stage and sex were important (Tables 2 and 3). Nymphs took ≈ 158 s, females required on average 98 s, and males took 74 s to choose an arm. Response time to first choice was not influenced by the treatment, nor was there a significant insect stage \times treatment interaction (Table 3). Treatment had a significant effect on the percentage of insects that walked ATW to the end of the Y-tube, but insect stage and the insect \times treatment interaction did not (Tables 2 and 3). Regardless of the insect stage, the visual + volatile cue combinations resulted in the greatest percentage of insects going ATW to the end of the Y-tube (Table 2). A lesser percentage of insects walked ATW to the end of the Y-tube in the presence of either visual or volatile cues.

Discussion

For most herbivorous insects, visual and/or volatile plant cues play a central role during host location. This fact has been exploited, with varying success, in monitoring and trapping programs for insect pests such as *R. pomonella* (Prokopy et al. 1987, 1990, Rull and Prokopy 2003, 2005), *Popillia japonica* Newman (Klein 1981), and *Diabrotica barberi* Smith and Lawrence (Ladd et al. 1984, Metcalf and Lampman 1989), just to name a few. In some cases, trap catch increased many-fold when these two host-locating modalities were combined (Wallbank and Wheatley 1979, Tuttle et al. 1988, Teulon et al. 1999). In general, however, we know very little about how these two modalities interact during host location. Most emphasis has been placed on the role of volatile cues, or in the case of homopterans, on visual cues. On closer examination, both cues are almost always involved (Pettersson 1993, Eigenbrode et al. 2002, Raguso and Willis 2002, 2005, Jiménez-Martínez et al. 2004), albeit perhaps to a lesser extent. The addition of the second modality may produce only subtle differences in response; nevertheless, understanding or being aware of these differences could be crucial in the development of an effective trapping device. Little information currently exists on the plant cues that influence host location in *L. hesperus*, but the increasing importance of this insect in recent years has led to a more urgent need for an efficient monitoring and trapping system.

In a previous study (Blackmer et al. 2004), we showed that immature and female *L. hesperus* were attracted to odors associated with alfalfa and that this response was influenced by plant phenology, conspecific odors, and/or by *Lygus*-induced feeding damage. However, the preference by adult females was much less than that of immature *L. hesperus* and males were not attracted to any of the plant or plant + conspecific treatments. We speculated that visual cues might be important during host location. One

study with *L. hesperus* (Landis and Fox 1972) and several studies with *L. lineolaris* (Prokopy et al. 1979, 1982, Boivin et al. 1982, Legrand and Los 2003) seem to support this conclusion. No previous studies have examined the response of *L. hesperus* when presented visual and volatile plant cues either singly or in combination.

Similar to our previous findings (Blackmer et al. 2004), we concluded that the responsiveness of *L. hesperus* to alfalfa odors was influenced by developmental stage and sex. When presented only volatile plant/conspecific cues, nymphs were less likely to exit the release tube, took longer to exit, were less likely to walk upwind, and took longer to make a choice between arms of the Y-tube than adults. However, nymphs that walked upwind were again better at selecting the arm of the Y-tube that contained the plant/conspecific treatments when only volatile cues were presented. Females and males exhibited similar orientation behaviors as they walked upwind, except males required less time to make their first choice and never preferred the plant/conspecific treatments when presented only volatile cues.

When the visual cue (LED) alone was presented to nymphs, it took longer for them to exit the release tube and a smaller percentage walked upwind, but there was a preference for the LED that was approximately equivalent to the response exhibited when volatile cues alone were presented. For the percentage of nymphs that walked ATW to the end of the Y-tube, however, there was a 3.1-fold synergistic increase in response relative to the percentage that walked ATW when volatile cues alone were presented. These findings show that both visual and volatile cues play a role in orientation behavior of nymphs but that volatile cues may be more important from a distance in initiating upwind orientation.

When nymphs were presented visual and volatile plant cue combinations, upwind orientation to previously preferred treatments (FA + C, VA + FD, FA + FD; Fig. 1) was not enhanced; however, for one marginal treatment (FA + FD/C), there was a 1.3-fold additive increase in attractiveness with the cue combination. In the first instance, the visual and volatile information was probably redundant, but in the latter case, when appropriate volatile information was apparently lacking, the response to the two cues was additive. For the percentage of nymphs that walked ATW, there was a 2.3- to 9.1-fold synergistic increase in responsiveness with the combination treatments relative to volatile cues alone. This was most likely a close-range response as similar response levels were not observed further downwind from the source (i.e., at the release site).

For females, when the visual cue was presented alone, there was a 1.3-fold additive increase in upwind attraction relative to volatile cues alone, and for the percentage that walked ATW, there was a 2.0-fold additive increase in response with the visual cue. For males, there was a nonsignificant 1.5-fold increase in upwind response relative to volatile cues alone, and for the percentage that walked ATW, there was a

2.2-fold synergistic increase in responsiveness to the LED. No significant differences in percentage of bugs exiting, time to exit, and percentage walking upwind were detected for either sex relative to the visual cue, suggesting that this cue may be more important from a distance for adults compared with nymphs.

For females, when visual and volatile cues were presented together, there was only a slight increase in upwind response for previously preferred treatments (VA + C and FA + FD; Fig. 2); however, for the previously marginal treatments (VA and FA + C), there was a 1.8- to 2.0-fold additive increase in attractiveness with the cue combinations. Males were more likely to walk upwind when visual and volatile cues were presented together (a 1.6- to 2.8-fold increase in response), but for the marginal hosts (FA and VA; Fig. 3), this difference was still not sufficient to obtain a significant preference. In the case of the increased attraction that males showed to VA + C, we cannot rule out the possibility that the increase responsiveness of the males may have been caused by the production of a sex pheromone by females in this treatment. For the percentage of females and males that walked ATW, there were 1.4- to 7.4-fold and 2.5- to 7.4-fold synergistic increases in responsiveness with the combination treatments, respectively. Evidently, visual cues were important to both sexes, but the enhanced attraction was still insufficient to obtain an adequate response from males.

Until now, no one has examined the effect of the combination of these cues on *Lygus* or any of the closely related species of mirids. However, a considerable volume of literature exists for the response of mirids to plant and conspecific volatiles. Several of these studies involved the use of gas chromatography-electroantennographic detection (GC-EAD) techniques; however, electroantennograms (EAGs) only indicate that the insect perceives the volatile compound and not how, or even whether, the insect will actually respond in a meaningful way to these compounds. With that caveat in mind, Chinta et al. (1994) tested the antennal response of *L. lineolaris* to insect-produced butyrates and synthetic plant compounds. They found that males were more responsive to two of the butyrates [hexyl butyrate and (*E*)-2-hexenyl butyrate] than females, but females were more responsive to the plant monoterpene, geraniol, than males. Groot et al. (1999) reported a similar difference between male and female EAG responses in the green capsid bug, *Lygocoris pabulinus* (L.) Males were more sensitive to a number of esters and females were more responsive to the plant compounds tested. Thus, for these two mirids, it would seem that there is a sexual dimorphism in responsiveness to various compounds, with males being more responsive to the insect-produced butyrates that are thought to play a role in mate location (Drijfhout et al. 2002, 2003, Ho and Millar 2002) and females being more responsive to plant compounds that probably play a role in host location and selection. These findings are similar to ours where we found behavioral differences in responsiveness for the sexes to plant/conspecific odors (Blackmer et al.

2004). Females were always more responsive to plant volatiles than males, which were more likely to be repelled by alfalfa odors in the absence of any female-associated cues. In this study, these responses were modified greatly, in some cases, by the addition of visual plant cues.

In nature, these two cues occur together, although they may not be perceived equally well depending on the environmental conditions and habitat. Volatile profiles of plants can vary because of time of year (Tiberi et al. 1999), time of day (Agelopoulos et al. 2000, Pecetti and Tava 2000, Rodriguez-Saona et al. 2001, Blackmer et al. 2004), cultural practices, and environmental conditions (Takabayashi et al. 1994, Gouinguene and Turlings 2002). Previous feeding damage by conspecifics or other herbivores can also influence headspace volatiles (Landolt et al. 2000, Rodriguez-Saona et al. 2003). This results in extremely complex and variable volatile profiles, from which the herbivore must select appropriate signals that stimulate the host location behavioral sequence. Visual cues, however, are not influenced by air movement and thus are effectively omnidirectional and stable over short distances from the source (Miller and Strickler 1984, Prokopy 1986). However, plant stress, leaf maturity, nutritive condition, foliage density, angle of illumination, and background may influence the plants visual appearance (Prokopy and Owens 1983). Although in our study these two modalities seemed to be redundant in a few cases, sensory system redundancy would be of selective advantage. If one system failed, suffered injury, became habituated or declined in efficiency with age, the insect might still be able to locate its host plant. Prokopy and Owens (1983) speculated that visual traps might prove more valuable in the case of visual specialists, but at least in this case, the addition of visual cues in any trapping system developed for *L. hesperus*, which is most likely a visual generalist given its host range, will probably lead to increases in trap catch efficiency. Our study suggests that, for males, visual cues may also be an important consideration in any trap design when the "bugs" are ultimately worked out for the sex pheromone of this species. Additional studies will focus on refining the upwind response of male and female bugs by examining trap characteristics such as trap type, trap contrast, height, shape, size, and the angle of landing surface. We expect that trap positioning relative to existing crops will also be an important consideration as we attempt to limit the movement from unaffected crops (i.e., alfalfa) to affected, but less preferred, hosts (i.e., cotton).

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